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Short Communication

# Toxin-free honey despite the alien *Senecio inaequidens* DC. invasion – a pilot study

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## Abstract

*Senecio inaequidens*, a fast-spreading perennial herb native to Southern Africa, is increasingly common in disturbed habitats across Europe, including urban areas. This species contains pyrrolizidine alkaloids (PAs), natural toxins harmful to humans and animals. In this pilot study, we investigated the potential for PA contamination in honey from hives located near large *S. inaequidens* populations in Prague, Czechia. Pollen analysis of honey, hive-stored pollen and honeycomb samples indicated a low and infrequent presence of Asteraceae-type pollen, which could be, but not definitively, attributed to *S. inaequidens*. Chemical analysis using liquid chromatography–mass spectrometry (LC-MS) detected trace amounts of the PA echimidin in three honey samples, likely originating from *Echium vulgare*. No other PAs were detected above quantifiable levels. Our findings suggest that, under current conditions, *S. inaequidens* contributes minimally to PA contamination in honey and does not present a significant health risk. However, potential shifts in flowering phenology warrant continued monitoring to assess future risks.

**Key words:** Health impact, narrow-leaved ragwort, non-native plant, pollen analysis, pyrrolizidine alkaloids

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## Introduction

Invasive species represent a substantial threat to global ecosystems – they degrade natural habitats, cause economic losses or influence human health (Pyšek et al. 2020; Bacher et al. 2024). A notable example in Europe is *Senecio inaequidens* (Asteraceae), an invasive plant that forms dense stands and spreads quickly along disturbed sites, such as roadsides and railways (Ernst 1998; Heger and Böhmer 2009; Vacchiano et al. 2013; Kaplan et al. 2018; Kutlvašr et al. 2024).

*Senecio inaequidens* DC., also known as Narrow-leaved Ragwort or South African Ragwort, is a perennial herb reaching a height of 30–100 cm. The plant has a shallow taproot; the stems are erect and branched from the base, covered by alternate and narrow leaves, ca. 2–10 cm in length and up to 1 cm in width. It usually flowers from June to November in Central Europe. The flowers are arranged in capitula with a diameter of 15–25 mm, forming a corymbose panicle. Each capitulum contains 10–15 male ray florets, characterised by a 5–8 mm long golden-yellow ligule and 80

bisexual disc florets. The fruit is a cylindrical achene, 3 mm long, with a short, dense covering of hairs (Hilliard 1977; Grulich 2004; Jäger et al. 2013; Kaplan et al. 2019).

It is native to southern Africa (Werner et al. 1991; Grulich 2004; Heger and Böhmer 2009). The first occurrence in Europe is associated with wool-processing facilities in the late 19$^{th}$ and early 20$^{th}$ centuries (Kuhbier 1977; Ernst 1998; EPPO 2006; Heger and Böhmer 2009). In the secondary range, it colonises various ecological habitats, encompassing dry to moist, stony to clay soils and exposed and partially shaded sites (Werner et al. 1991; Adolphi 1997). In its native habitat, it grows on grassy, steep slopes, but also on streambanks from 1400 to 2850 m a.s.l. (Hilliard 1977; Werner et al. 1991). Across Europe, it mainly occupies warm, sandy or gravelly disturbed soils along transportation corridors and in other open habitats, for example, abandoned industrial sites, quarries or logged areas (Werner et al. 1991; Hard 1993; Radkowitsch 1997; Mückschel 2000; Bornkamm 2002; Blanchet et al. 2015; Kaplan et al. 2018). However, it has also been observed to penetrate semi-natural habitats (Brandes 1999; Scherber et al. 2003), where it can compete with some native species (Quaglini et al. 2025). The consequences of climate change, such as mild winters and warm, dry growing seasons, may facilitate the further spread of this species in Europe (Nehring et al. 2013).

In addition to the ecological risk, *S. inaequidens* contains a set of pyrrolizidine alkaloids (PAs), natural plant toxins found in 3% of all flowering plants from a total of 13 families (e.g. Asteraceae, Boraginaceae, Fabaceae, Rhamnaceae) (Smith and Culvenor 1981). A total of nine PAs were found in *S. inaequidens* plants, for example, integerrimine, retrorsine, senecionine, seneciphylline, senecivernine and usaramine (Röder et al. 1981; Bicchi et al. 1985; Eller and Chizzola 2016). The highest concentration of PAs was found in young shoots and inflorescences (Eller and Chizzola 2016).

PAs themselves are not directly toxic to vertebrates. After ingestion, they are metabolised in the liver to reactive pyrrole derivatives that bind to DNA and proteins, causing liver cell death, progressive cirrhosis and eventually liver failure (Mattocks 1986; Johnson et al. 1989). Some PAs are known to be mutagenic and genotoxic (Rubiolo et al. 1992). Furthermore, the significant influence on the metamorphosis of *Hymenoptera* was also proven (Heneberg et al. 2025).

PAs can enter the human food chain most often through contamination of agricultural products by weeds or from food supplements and traditional medicinal products, typically some types of teas (BfR 2013b). Livestock can be intoxicated by direct ingestion of fresh plants and dried forage (Johnson et al. 1989; Stewart and Steenkamp 2001; Stegelmeier 2011; Wiedenfeld 2011). The PAs and their residuals are also transferred further to animal products, as has been reported for eggs (Edgar and Smith 2000) or milk (Panter and James 1990; Mulder et al. 2020; Kalač and Kaltner 2021).

The PAs are commonly reported also in honey (and foods containing honey) from the nectar and pollen of toxic plants (Edgar et al. 2002). Their quantities are sometimes very high, reaching tens to hundreds, sometimes even thousands of μg/kg of honey (Kempf et al. 2008, 2010, 2011; Dübecke et al. 2011; BfR 2013a; Griffin et al. 2013; Kast et al. 2014; EFSA 2016; Neumann and Huckauf 2016). Such honey poses a health risk, especially if honey containing PAs is consumed over a long period, leading to liver damage, fibrosis and increased risk of cancer (Mattocks 1986; Wiedenfeld and Edgar 2011). According to the

German Federal Institute for Risk Assessment, daily doses should not exceed 0.007 µg/kg body weight (BfR 2013a).

In our pilot study, we studied the content of PAs in honey and the presence of *S. inaequidens* pollen from hives situated near large invasion sites. The aim was to determine if PAs could be transferred to honey, thereby posing a health risk to consumers.

## Materials and methods

In this study, we examined the occurrence of pollen grains and the presence and concentration of pyrrolizidine alkaloids (PAs) in honey, hive-stored pollen and honeycomb from hives. The hives were situated at two localities in Prague (Czechia; hive 1–50.0504°N, 14.5348°E, hive 2–50.1237°N, 14.3934°E) where *S. inaequidens* is widespread (Fig. 1).

Five honey samples, one sample of pollen and one honeycomb sample from a hive were analysed. Furthermore, a reference sample of *S. inaequidens* flowers was collected to determine the pollen grains. The honey samples were collected at various times to encompass the entire growing season. Samples further referred to a form: hive month/year, for example, hive 1 VII/19. See Table 1 for details.

To confirm the presence of *S. inaequidens* pollen grains, all collected samples were analysed in December 2019. The samples were processed using a standard acetylation method (Moore et al. 1991). Pollen grains were identified using a light micro-

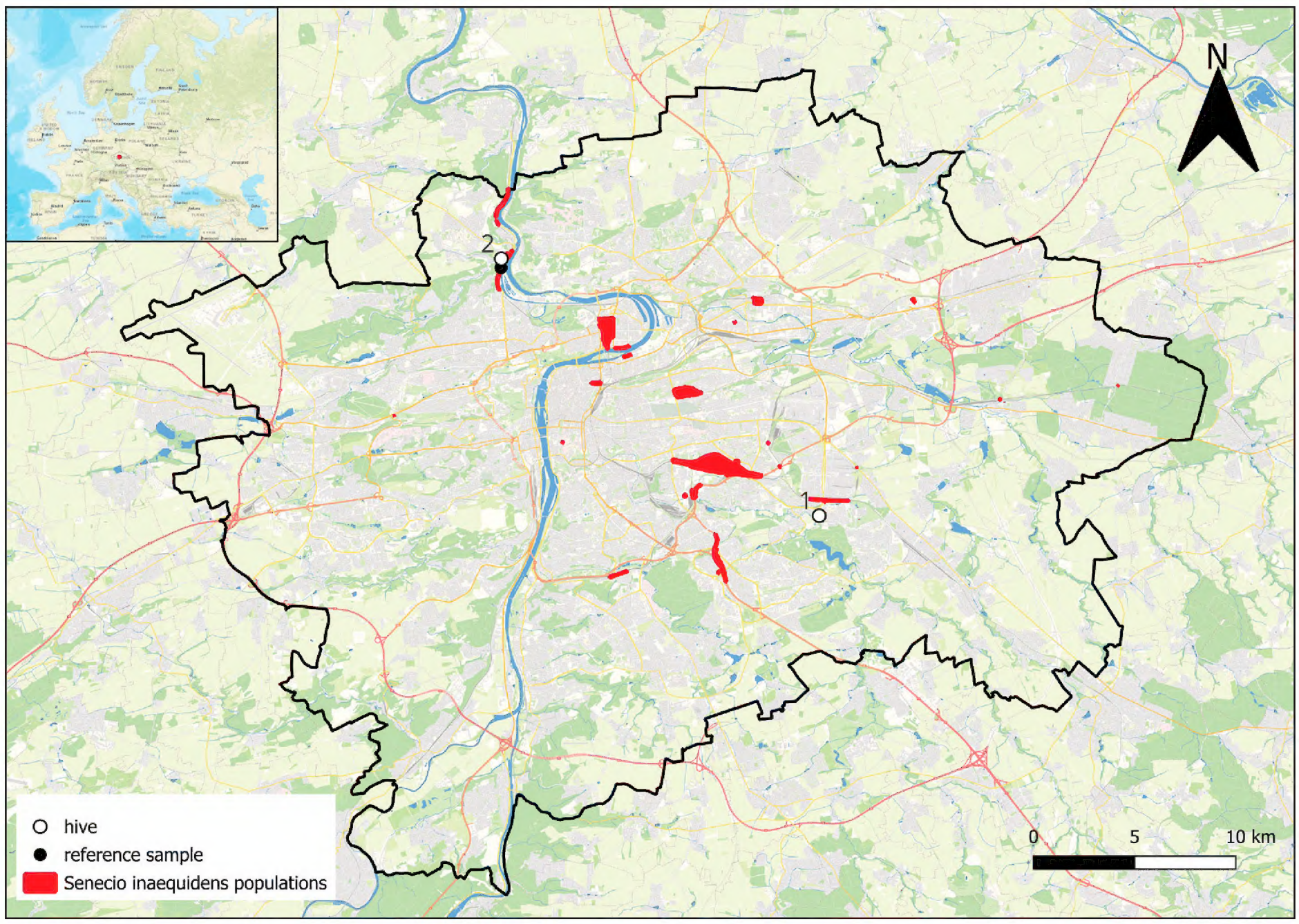


**Figure 1.** The map of study hives and distribution of *S. inaequidens* in Prague. The red polygons represent the populations of *S. inaequidens* (2019). White points indicate two hives located near large populations. The black point shows the location of the pollen reference sample collection.

**Table 1.** Overview of the investigated samples. The pollen samples were not undergoing PA analysis due to the low representation of Asteraceae/Senecio type in the pollen analysis found. See Supplementary Material 1 for protocols of PA analyses.

| Sample | Date of sampling | Pollen analysis | PA analysis | Type of material | Coordinates |
|---|---|---|---|---|---|
| hive 1 VIII/19 pollen | August 2019 | yes | no | hive-stored pollen | 50.0504°N, 14.5348°E |
| hive 1 VIII/19 combs | August 2019 | yes | no | honeycomb | 50.0504°N, 14.5348°E |
| hive 1 VII/19 honey | July 2019 | yes | protocol no. 31/20 | honey | 50.0504°N, 14.5348°E |
| hive 2 V/18 honey | May 2018 | yes | no | honey | 50.1237°N, 14.3934°E |
| hive 2 V/19 honey | May 2019 | yes | protocol no. 28/20 | honey | 50.1237°N, 14.3934°E |
| hive 2 VII/19 honey | July 2019 | yes | protocol no. 29/20 | honey | 50.1237°N, 14.3934°E |
| hive 2 VIII/19 honey | August 2019 | yes | protocol no. 30/20 | honey | 50.1237°N, 14.3934°E |
| pollen reference sample | August 2019 | yes | no | inflorescence | 50.1221°N, 14.3938°E |

scope and palynological keys (Moore et al. 1991; Beug 2004). We used this method due to the low number of samples and its robustness to possible contamination by non-pollen particles, whereas some other methods (e.g., metabarcoding) could show false positives and noise. As *S. inaequidens* pollen grains are not morphologically distinct from those of other species of the *Senecio* genus or similar Asteraceae (Beug 2004), we classified them under a general "Aster/Senecio" pollen type (Fig. 2).

The presence and quantitative content of PAs were determined using liquid chromatography coupled with mass spectrometry (LC-MS) in January 2020 (Crews et al. 2010; Kempf et al. 2011; Fuente-Ballesteros et al. 2024). A total of 29 different PAs of four honey samples were analysed, including PA-*N*-oxides (see Table 1 and Suppl. material 1 for laboratory protocols; Dzuman et al. (2020)).

## Results

### Pollen analysis

In total, seven samples were analysed. The honey samples had only a low concentration of pollen grains and crystallised sugar was visible. The Aster/Senecio pollen type was found only in one sample in small quantities (hive-stored pollen from hive 1 VIII/19). For details, see the pollen diagram in Fig. 3.

### Honey samples analysis

We investigated 29 PAs or their *N*-oxides that could originate from *S. inaequidens* or other species, but the analysis did not show any such alkaloids, except for echimidin. Echimidin was detected in three samples in low concentrations rang-

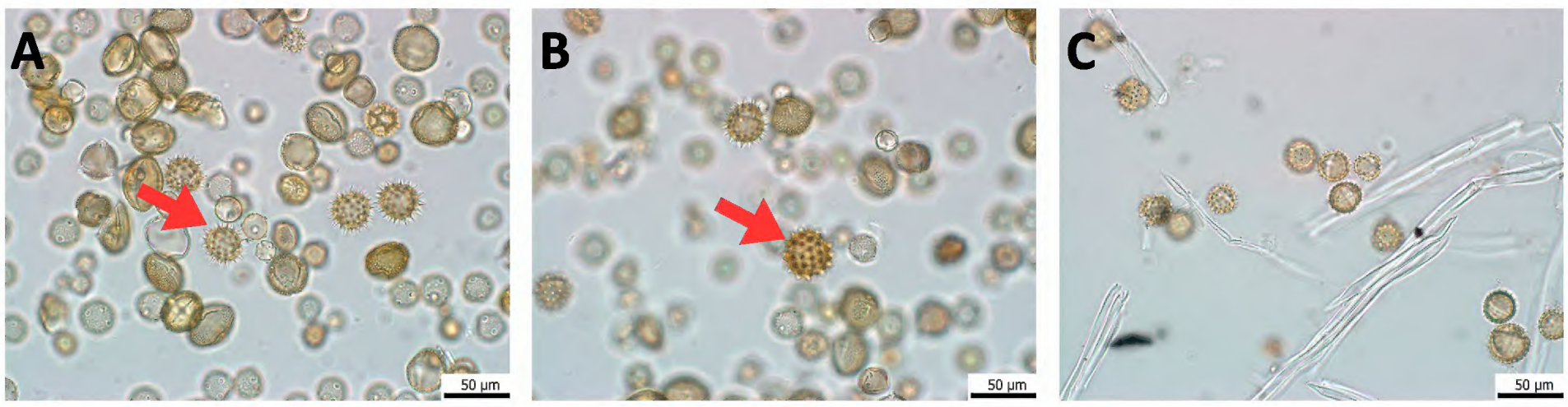


**Figure 2.** Possible occurrence of *S. inaequidens* pollen grains (marked by the red arrows) obtained from hive 1 VIII/19 pollen (**A, B**) and reference sample acquired from capitula of *S. inaequidens* (**C**).

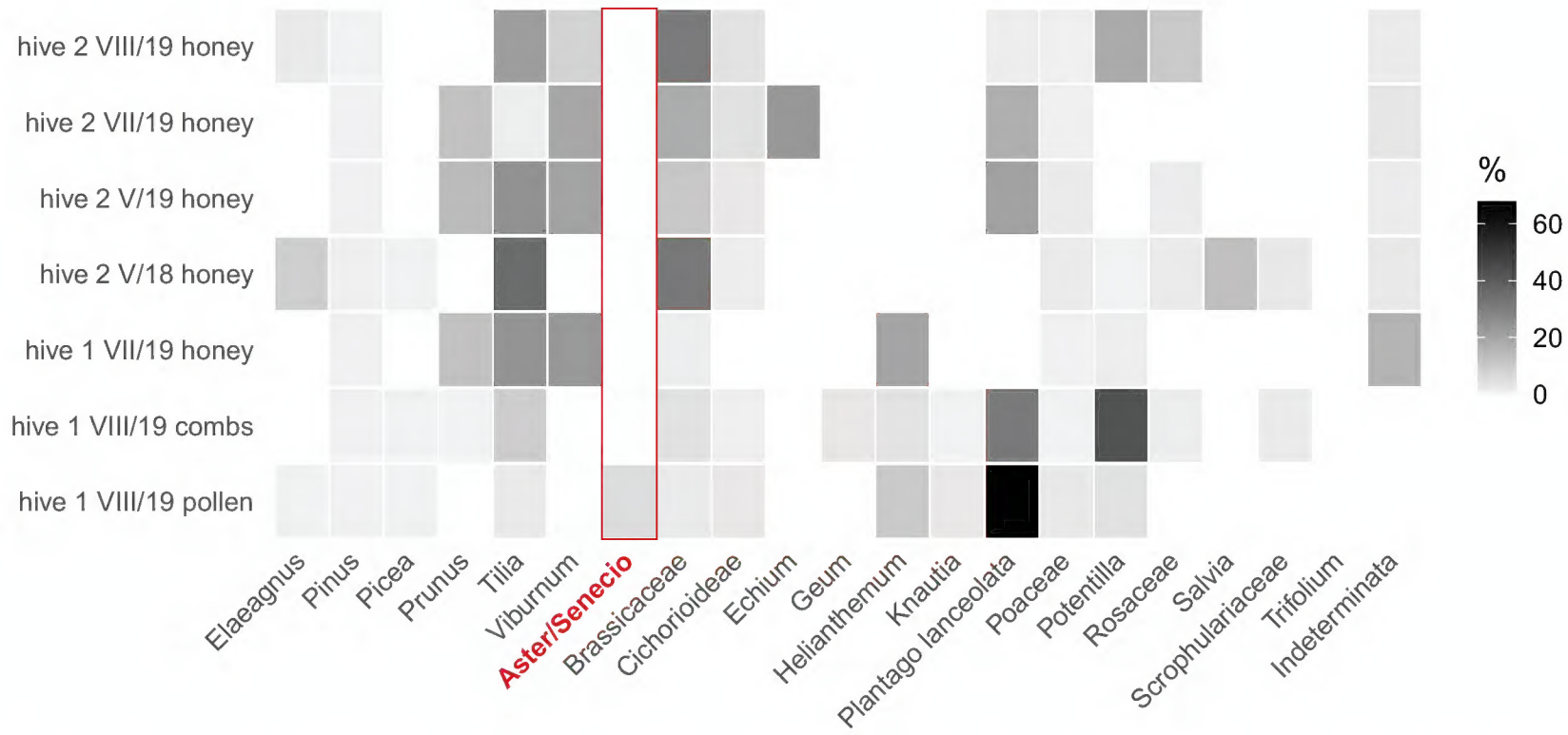


**Figure 3.** Pollen diagram of the samples, with the Aster/Senecio pollen type shown in red. The average proportion of pollen grains across samples was 331. The number of PA analysis protocols for the investigated sample is shown in Table 1.

ing between 1–7 μg/kg: 1 (p. no. 30/20), 2 (p. no. 31/20) and 7 (p. no. 29/20) (Suppl. material 1).

## Discussion

The probable pollen type corresponding to the *S. inaequidens* was detected only in the pollen sample in a very low concentration. The pollen type Aster/Senecio, which could also correspond to the species *S. inaequidens*, was occasionally detected in other samples, but due to the variability of the pollen type of the Asteraceae family, its further occurrence in the samples is unlikely (Fig. 3, Indeterminata group; Beug (2004); Halbritter and Auer (2021)). Based on the pollen spectrum (Fig. 3), we suppose that bees preferred honeydew from flowering woody plants as their main food source. The only comparable study that can be related to *S. inaequidens* is a study on *S. jacobaea* (Kempf et al. 2011), where minimal Senecio-type pollen in honey (0–6.3%) was found, aligning with our results.

All investigated PAs were below the limit of detection except for echimidin. In addition, echimidin was found only in low concentrations. The source of echimidin is most likely *Echium vulgare*, which was found to be abundant in the vicinity of the studied sites. This finding corresponds with pollen analysis (hive 2 VII/19), which also reveals the presence of *Echium* pollen type. *Echium vulgare* is considered to be one of the major reasons for PA contamination in honey (Dübecke et al. 2011; Kempf et al. 2011). We must also add that nectar appears to be a more significant source of PAs in honey than pollen in the case of *E. vulgare* (Lucchetti et al. 2016).

As regards PAs in honey originating from *S. inaequidens*, the risk of contamination will increase significantly if *S. inaequidens* starts flowering earlier than before. In early spring, other sources of honeydew and pollen are scarce and bees may use alternative sources such as *S. inaequidens*. Over the past twenty years, S. inaequidens has begun to bloom in June or July (Grulich 2004; Jäger et al. 2013; Kaplan et al. 2019), whereas in recent years, it has already been blooming in May (Heneberg et al. 2025). In warmer parts of Central Europe, still-flowering overwintering stems have

already been observed in February (Karlík et al. 2020; Suppl. material 2). Autumn flowering, on the other hand, may not pose as a significant risk as many beekeepers leave the honey from the last honey flow with the bees to help them overwinter and, therefore, it does not enter circulation (Sammataro and Avitabile 1998).

Not only the amount, but also the distance of the forage from the hive or the landscape structure can play a significant role. Bees typically use resources from up to about 3 km, but if the forage is dense enough, they usually travel shorter distances (Beekman and Ratnieks 2000; Danner et al. 2016). These studies suggest that the location of hives for studying the content of PAs in honey and pollen should be in the immediate vicinity of the population of the studied species, so that it is offered to the bees as the most easily accessible forage.

In conclusion, we did not find a significant amount of pollen grains in the honey over the season, nor did we find the PAs originating from *S. inaequidens*. Even though the hives were located close to large populations of *S. inaequidens*, the pollen/PAs content in the analysed samples can be described as negligible, not posing a health risk to humans. However, it can be stated that the current flowering phenology of *S. inaequidens* minimises the potential negative impact on human health. As the phenology is slowly changing, the PAs and pollen in honey need to be monitored.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Artificial Intelligence (AI) use

The following AI tools were used in the preparation of this manuscript:

**Description:** Using Grammarly to edit text and language.

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### Author contributions

Conceptualization: PK. Data curation: PK, LP. Formal analysis: LP. Methodology: PK. Supervision: JP. Validation: JK. Visualization: PV, KK. Writing – original draft: KK, JK. Writing – review and editing: JP, PV.

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## Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Supplementary material 1

### Supplementary information 1

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Data type: pdf

Explanation note: Original laboratory protocols covered 29 PAs or their *N*-oxides in honey samples that could originate from *S. inaequidens*. Výsledek=Result, Jednotky=Units, Zkušební metoda=-Method.

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Link: https://doi.org/10.3897/neobiota.106.175238.suppl1

## Supplementary material 2

### Supplementary information 2

Authors: Petr Karlík, Josef Kutlvašr, Klára Kušková, Libor Petr, Pavla Vachová, Jan Pergl
Data type: jpg
Explanation note: Overwintering stems of *S. inaequidens* that were observed blooming in February 2020.

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